PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publicati n Number:
A61K 39/35, C07K 15/28	A1	WO 94/14475
		(43) International Publication Date: 7 July 1994 (07.07.94)

(21) International Application Number: PCT/US93/12501

(22) International Filing Date: 20 December 1993 (20.12.93)

(30) Priority Data:
07/994,126 21 December 1992 (21.12.92) US

(71) Applicant: TANOX BIOSYSTEMS, INC. [US/US]; 10301 Stella Link, Houston, TX 77025 (US).

(72) Inventor: CHANG, Tse, Wen; 3000 Bissonet, Apartment 7110, Houston, TX 77005 (US).

(74) Agent: MIRABEL, Eric, P.; Tanox Biosystems, Inc., 10301 Stella Link, Houston, TX 77025 (US).

(81) Designated States: AU, BB, BG, BR, CA, FI, HU, JP, KP, KR, LK, LU, MG, MN, MW, NO, PL, RO, SD, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ALLERGEN-SPECIFIC IgA MONOCLONAL ANTIBODIES AND RELATED PRODUCTS FOR ALLERGY TREATMENT

(57) Abstract

Disclosed are pharmaceutical preparations which include as their essential ingredient human monoclonal IgA or IgG antibodies, specific for major allergenic proteins found in ragweed, house dust mites, and cat and dog dander. Also disclosed are conjugates comprising physiological compatible polymer backbones or microbeads and a plurality of covalently conjugated allergen-specific binding molecules. Suitable binding molecules can be IgG or IgA, or their $F(ab')_2$, Fab, or Fv fragments, specific to allergenic proteins, such as those mentioned above. Also disclosed are methods for treating a patient with allergic rhinitis, asthma, or conjunctivitis by applying a pharmaceutical preparation containing the antibodies or conjugates specific for the allergenic molecules to which the patient is sensitized, to the patient's affected mucosal tissues, such as the nasal linings, the respiratory tract, or the eyes.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

ALLERGEN-SPECIFIC IgA MONOCLONAL ANTIBODIES AND RELATED PRODUCTS FOR ALLERGY TREATMENT

Field of Invention

The invention relates to allergen-specific IgA and IgG monoclonal antibodies and related products, and their use in diagnosis, allergen purification and in the treatment of IgE-mediated allergies.

Background of the Invention

Immunoglobulin E (IgE) is the agent primarily responsible for causing type-1 hypersensitivities. This type of hypersensitivity is manifested as any of a number of common disease conditions, including allergic rhinitis, allergic asthma, and allergic conjunctivitis. The mechanism of type-1 hypersensitivities in patients with IgE-mediated allergies stems from the fact that they synthesize allergen-specific IgE. The IgE circulates in the blood and binds to high-affinity IgE.Fc receptors (FcεRI), which are on basophils in the circulation and on mast cells in various tissues. Allergens enter the body through inhalation, ingestion, or through the skin. The allergen molecules bind to the binding sites (Fab) of IgE which is on the surfaces of mast cells and basophils, aggregate the underlying FcεRI, and trigger the release of histamines and other pharmacological mediators, causing the well-known allergic symptoms.

The tissues that are most susceptible to local IgE-mediated allergic reactions are the nasal linings (in patients with allergic rhinitis), the

mucosal linings of bronchial tracts (in patients with allergic asthma), and the mucosal surfaces of the conjunctiva of the eyes (in patients with allergic conjunctivitis). This local sensitivity arises because the allergens enter the respiratory tract through inhalation and get trapped on the mucosal surfaces of the nasal linings and bronchial tracts of the respiratory airway. The eyes and ears are also susceptible to allergen entry and local allergic reactions. Airborne allergens contact with the moist surfaces of the eyes or ears and are retained by the mucous tissues.

Mast cells densely populate the mucosal tissues that are exposed to the exterior environment. The allergens that are taken up by the mucosal epithelial cells and transported across the cells to the interstitial spaces bind to the IgE on these mast cells before entering the circulation.

Although IgE can cause annoying, discomforting, and sometimes, severe and life-threatening symptoms in affected individuals, it exists *in vivo* only in minute quantities. Although the levels of IgE in serum are generally higher in atopic than in normal individuals, the total amount of IgE in the body is estimated to be less than 1 mg in most individuals. Thus, the immune mechanism mediated by IgE and the sensitization of mast cells and basophils is a sensitive biological system, which requires only minute quantities of antigens/allergens to induce serious allergic reactions. For an individual highly sensitive to bee venom, for example, the sting of a bee can cause an anaphylactic shock or even death, although

the allergenic proteins are only a minor chemical components in the *Hymenoptera* venom. Or, as another example, a small numbers of pollen particles or animal dander particles are capable of triggering the allergic reactions in the nasal linings and in the airway of sensitized individuals.

In the usual situation, the allergens which enter the body of a person are not in their pure form. The allergenic molecules in pollen particles, house dust mites, and animal dander that are breathed in are relatively minor components of the whole particles. For example, the content of the main allergenic protein, antigen P_1, of dust mite *Dermatophagoides pteronyssinus* (*Der p* I) in a household highly infested by the mites, accounts for about 10-20 μg per g of dusts. Lau-Schadendorf, S. *et al.*, *J. Allergy Clin. Immunol.* 87:41-47 (1991). The allergenic protein is a cysteine protease contained in the fecal excretion of the mites.

The allergenic substances employed in diagnosis and immunotherapy are almost always extracts of allergenic substances, such as grass or tree pollens, house dusts, cat and dog dander. Because of the crude nature of these extracts, the International Union of Immunological Societies of the World Health Organization established the Allergen Standardization Subcommittee to oversee the standards of production, packaging, and preliminary testing of the allergens. For a number of allergenic substances, the standard tests are assays that quantify the major

allergenic proteins in the extracts.

Over the past several years, increasing efforts have been made to identify and purify the major allergenic proteins from increasing numbers of allergenic substances. For example, the major allergen *Can f* I (antigen 13) was purified from dog dander. De Groot, H., *et al.*, *J. Allergy Clin. Immunol.* 87: 1056-1065 (1991). Several academic laboratories have been able to provide immunochemical assays for allergen-specific IgE and IgG using purified allergen proteins as the antigens in these assays. Hamilton, R.G. and Adkinson, Jr., N.F. in *Manual of Clinical Laboratory Immunology*, eds. Rose, N.R. *et al.*, pp.689-701, 4th ed., American Society of Microbiology, Washington, D.C. (1992); Hamilton, R.G. and Adkinson, Jr., N.F. ibid., pp.702-708. These purified allergenic proteins and the establishment of the immunochemical assays for human allergen-specific IgG are useful for the identification of B cell clones and monoclonal antibodies specific for the allergens.

More recently, the genes encoding several major allergenic proteins have been cloned. For example, the cDNA for the major mite antigen, *Der p* I. Chua, K.Y. *et al.*, *J. Exp. Med.* 167: 175-182 (1988), and the cDNA for the major allergen in cat saliva, *Fel d* I. Morgenstern, J.P. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88: 9690-9694 (1991), have been cloned. The cloning of these genes enables their expression and the production of the recombinant proteins.

Although the synthesis of allergen-specific IgE is the primary determinant of IgE-mediated hypersensitivities, it is not the only factor. Persons who are exposed to but not sensitized to an allergen are known to produce IgG specific to the allergen. It is believed that these allergen-specific IgG serve as protective, blocking antibodies. People with IgA deficiency are more prone to develop IgE-mediated allergies, suggesting that IgA secreted to the mucosal surfaces of the respiratory tract can neutralize or block the trapped allergen particles or molecules, and inhibit their entry into the tissue.

In desensitization immunotherapy, which is widely used, patients are immunized with small amounts of allergens over an extended period. While this therapy is effective in alleviating symptoms in about half of the allergic patients, its mechanism of action is not well understood. One hypothesis is that the treatment induces IgG blocking antibodies, because elevated levels of these antibodies can be detected in the treated patients.

The treatment of allergies has not changed substantially over the past eighty years. Desensitization immunotherapy is the primary therapy for the patients with allergic rhinitis. Immunosuppressive drugs, such as steroids, for suppressing immune activities, and bronchial dilators, such as α-albuterol, for relieving asthmatic symptoms, are the primary treatments for patients with allergic asthma. The medical advances have been limited to improving classification of the allergenic substances,

improving the diagnostic methods for determining a patient's allergen profile, and improving the control of and expanding the library of allergen extracts for immunotherapy. On the research side, progress has been made in the identification and the isolation of major allergenic molecular components in allergenic substances. For example, the chief protein components in ragweed (the most important allergen in causing hay fever in the fall season), in house dust mites (the most important allergen in causing allergic asthma), and in cat and dog dander and saliva, have been identified and isolated. The genes encoding several major allergens have also been cloned.

Thanks to the development of methodologies for immortalizing human B lymphocytes using cell fusion or transformation with Epstein-Barr virus (EBV), it is possible to identify human monoclonal antibodies with antigen specificity of interest. The more recent development of methodologies to construct recombinant phage incorporating human V_H and V_L libraries, to express the combinatorial V_H/V_L, and to screen the expressed antibody fragments for antigen specificity have added another powerful tool in the identification of antibody species of interest. In addition, the improvement in gene transfection and expression of antibody genes in myeloma or other cell lines have enabled the production of human monoclonal antibodies in large quantities. The use of allergen-specific antibodies as an allergy therapy appears promising and viable.

Summary of the Invention

The invention includes allergen-specific IgA and IgG, preferably monoclonal IgA or IgG, and more preferably human monoclonal IgA and IgG, and use thereof in the treatment of IgE-mediated allergic diseases, such as allergic rhinitis, allergic (extrinsic) asthma, and allergic conjunctivitis, by employing antibodies to inhibit the entry of allergenic molecules into mucosal tissues ("immune exclusion"). IgA antibodies are preferred because they are more resistant to proteolytic cleavage by proteases in the mucosal fluids. The invention also includes allergen-specific IgA or IgG or the antigen-binding fragments of these antibodies, such as $F(ab')_2$, Fab, or Fv, conjugated to polymer backbones or microbeads. These polymers are non-allergenic and non-toxic, and with proper modification can have many antibody molecules conjugated to them. Examples of suitable polymers include dextran, agarose, and cellulose. The preferred microbeads are made from the cross-linking of these polymers. The polymerized conjugates may have the advantage over unconjugated antibodies or fragments in that they are less likely to be absorbed by the mucosal epithelial cells and more likely to be cleared along with the mucous secretion.

The allergen-specific IgA or IgG, or the related constructs, are to be applied in a pharmaceutical solution or suspension to the mucosal tissues of patients who are allergic to that allergen. The solution or

suspension can include water, preservatives, surfactants and other standard ingredients. The antibodies bind to the allergenic protein (or to the other molecules to be targeted) that is released from the inhaled particles. This binding inhibits the allergen from being taken up by the mucosal epithelial cells and hence inhibits allergic symptoms.

The preferred pharmaceutical preparation of the allergen-specific IgA or IgG antibodies, or the related constructs, is together with a physiological buffer, suitable for application by dropper. About 1-2 drops are applied per nostril, eye, or ear to effect treatment thereof. The concentration of the active ingredient in the solution is in the range of 20 to 1000 μg per ml, or about 1 to 50 μg per drop.

The active ingredient may also be prepared in a solution for administration with a metered dose inhaler, in order to reach mucosal tissues at the lower end of the respiratory tract. The concentrations would be in the same range as noted above, and the solution may be applied every 2 to 4 hours. It may also be applied with a decongestant medication, if desired.

The allergen-specific IgA or IgG, or their conjugates with polymers and microbeads, may also be used in manufacturing allergenic substances, which may be used diagnostically or for desensitization immunotherapy. The allergen-specific substances of the invention can be adsorbed onto an affinity column and used for purifying the specific

allergens from crude extracts of allergenic substances, such as ragweed, house dusts, and cat and dog dander. The purified allergens are better antigens than the crude extracts for diagnostic uses, *i.e.*, to analyze whether a patient is allergic to that particular allergen, and for desensitization immunotherapy. The purified allergen-specific IgA and IgG, or the conjugates, can also be used as standards in diagnostic assays which measure the levels of allergen-specific antibodies in patients. This determination is useful to decide if the patient is a candidate for desensitization immunotherapy, and also to determine whether the therapy is succeeding.

The allergens which would typically be targeted by the substances of the invention include *Amb a* I, the dominant allergen in short ragweed (*Ambrosia elator*), *Der p* I and *Der f* I, the major allergens from house dust mite species (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), *Fel d* I, the major allergen from the saliva of house cats (*Felis domesticus*), and *Can f* I, the major allergen from the dander of domestic dogs (*Canis familiaris*).

These allergens are preferably targeted with monoclonal antibodies. The monoclonal antibodies are produced by B cells from patients who have natural sensitivities to the allergens or who have been immunized with the allergens in desensitization immunotherapy. The B cells from these donors are immortalized by fusion with myeloma cells, such as NSO

or Sp2/0 cells, or transformed with EBV. The antibodies secreted by these immortalized cell lines are screened by ELISA or comparable immunochemical assays for specific reactivity with the allergens of interest. The V_H and V_L of antibody fragments may also be identified with the combinatorial V_H/V_L library expression and screening methodologies, as described further below.

To make IgA antibodies of the invention, the genomic DNA fragment containing the segment encoding V_H domain of an antibody of a desired specificity is linked to the genomic DNA fragment containing segments encoding the constant domains (CH1, CH2 and CH3) of human α chain. The genomic DNA fragment of the V_L is linked to the genomic DNA fragment of human κ or λ light chain. The recombinant DNA species in their appropriate plasmids are then used to transfect cells of a nonproducer myeloma cell line, such as NSO or Sp2/0. The procedures for the construction of the composite DNA fragments, the transfection of the recipient cell line with the DNA, and the screening of the transfectants for production of monoclonal IgA antibodies specific for the allergen/antigen are similar to those procedures used for preparing transfectoma cell lines producing chimeric antibodies, and are well known in the art.

Detailed Description of the Invention:

The small quantities of allergenic molecules which are present in the environment, and the small quantities of IgE present in the body, suggest that an effective therapy that employs specific antibodies to avoid or block the entry of allergenic molecules into the mucosal tissue would require only small quantities of these antibodies. However, the allergenic molecules must be accessible by the allergen-specific antibodies so that their entry to the mucosal tissue can be successfully inhibited. The allergenic proteins should be soluble in aqueous medium and in the mucous fluids, once they are released from the particles. The mucous contains a host of hydrolytic enzymes that should aid in digesting and loosening the contents of the particles. In any event, one can assume that the mucosal epithelium does not take up the whole pollen or mite particles and that only the released, soluble allergenic molecules cause the allergic reactions.

The hypothesis that pollen particles or house dust particles release the crucial allergenic proteins into the mucous fluids can readily be tested. For example, a sample of collected house dusts can be incubated with nasal mucous secretion *in vitro* for varying lengths of time (1 minute to 30 minutes). The mixture is then centrifuged to pellet particles. The content of *Der p* I protein in the supernatant is then measured by a standard immunochemical assay for *Der p* I (see below). The higher the level, the more effective the therapy of the invention is likely to be.

As noted above, although allergic individuals make IgE against the allergenic substance, the levels of such allergen-specific IgE are very low. Thus, to make allergen-specific IgA or IgG antibodies, one could isolate the B cell clones expressing the allergen-specific IgE, isolate the V_H and V_L gene segments, and then use these segments to construct IgA or IgG monoclonal antibodies. One could then use conventional technology to make chimeric antibodies.

However, the levels of allergen-specific IgE in allergic patients is low, making isolation of allergen-specific IgE-producing B cells difficult. As noted above, however, it is known that patients who are sensitized to an allergenic protein synthesize not only allergen-specific IgE but also allergen-specific IgG. The levels of the allergen-specific IgG are much higher than those of the allergen-specific IgE. Ishizaka, K. in *Allergy: Principles and Practice*, eds. Middleton, Jr., E., *et al.*, pp. 52-70, 3rd ed., The C.V. Mosby Co., St. Louis (1988). As reported by Hamilton, R.G. and Adkinson, Jr., N.F. in *Manual of Clinical Laboratory Immunology*, eds. Rose, N.R. *et al.*, pp. 702-708, 4th ed., American Society of Microbiology, Washington, D.C. (1992), in patients who have received desensitization immunotherapy by the repetitious immunization with the allergens, the allergen-specific IgG levels are much higher than those in unimmunized patients. Thus, patients immunized with allergens are a logical source to provide lymphocytes for the isolation of allergen-

specific IgG-producing B cell clones and/or antibody gene.

One practical source of allergen-specific IgG-producing B cell clones is from patients who have sensitivity to an allergen and who have been or are being immunized with the allergen. These patients can be identified by the allergist/physician treating them. The extent of improvement resulting from the immunization indicates that the patient has made produced a considerable immune response to the allergen/immunogen. The levels of allergen-specific IgG in those patients can be quantitatively determined using assays for allergen-specific IgG. *See* Hamilton, R.G. and Adkinson, Jr., N.F. in *Manual of Clinical Laboratory Immunology*, eds. Rose, N.R. *et al.*, pp. 702-708, 4th ed., American Society of Microbiology, Washington, D.C. (1992). The peripheral blood from these patients can be used as the source for allergen-specific IgG-producing B cells, from which the genes encoding for the V_H and the V_L of the allergen-specific IgG can be isolated.

Established methods for immortalizing human B cells by EBV transformation or by fusion with myeloma cells (such as NSO cells), or by the combination of these procedures, can be used for making immortalized cells. The EBV transformation procedure for making B cell clones was recently summarized in step-by-step format by Tosato, G. in *Current Protocols in Immunology*, eds. Coligan, J.E., *et al.*, § 7.22.1, John Wiley and Sons, New York (1991). Similarly, the step-by-step

procedure for preparing hybridomas, which can be used on murine or human lymphocytes, is described by Yokoyama, W.M. *ibid.* at § 2.5. These procedures also describe methods for screening the immortalized B cell clones to determine if they secrete antibodies with the desired antigen specificity, for the sequential subcloning of the B cell clones, and for the production of the antibodies. **Example 2** describes the design and use of enzyme-linked immunosorbent assays (ELISAs) for the detection and measurement of allergen-specific IgG and IgA.

An alternative method to identify and isolate of the genes encoding the allergen-specific antibodies is to employ the "V_H/V_L combinatorial library" methodology. The B cells from the peripheral blood of donors are first isolated. The individual V_H and V_L libraries are prepared by polymerase chain reaction (PCR) techniques using degenerate oligonucleotide primers covering the 5' ends of the V regions. The V_H and V_L fragments are then incorporated into one expression vector, in order to be coexpressed and to form antibody binding domains.

Several expression systems which use unique screening procedures have been developed. A method that expresses the combined V_H/V_L library in bacteriophage λ vector and presents it in the form of Fab fragments in the lysates of *E. coli* colonies (for subsequent immunochemical screening procedures) was described in detail by Barbas, C.F. and Learner, R.A., in *Methods: Companion Methods Enzymol.* 2:

119-124 (1991). A method which expresses the V_H/V_L on the surface of a filamentous phage fd, which can be isolated with antigen-coupled affinity matrices, was described by McCafferty J. *et al.*, in *Nature* 348: 552-554 (1990).

For achieving the biological effect of excluding inhaled or contacted allergens, both allergen-specific IgA and IgG will function well. However, since IgA is the dominant isotype at the mucosal surfaces, the preferred allergen-specific antibodies for mucosal applications are IgA isotype. The methods for cloning the restriction enzyme genomic DNA fragments containing V_H and of V_L of allergen-specific IgG antibodies, linking those fragments to the genomic DNA segments of human α and κ (or λ), the transfection of the DNA into non-producer myeloma cells, such as NSO or SP2/0 cells, the screening of the transfectants for the secretion of allergen-specific IgA, and the sequential cloning for the selection of stable secretors, are carried out in the same manner as for preparing transfectomas secreting chimeric antibodies. These methods have been described by various investigators in detail, and are well known in the art. *See, e.g.*, Morrison, S.L. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 81: 6851-6855 (1984); Liou, R.S. *et al.*, *J. Immunol.* 143: 3967-3975 (1989).

IgA exists in both monomeric and dimeric forms. The IgA in circulation is mostly monomeric, whereas the IgA secreted to the mucous

or other external fluids is mostly dimeric. The dimeric form has an extra J chain (15,000 daltons) and a secretory component (70,000 daltons), both of which are linked covalently to the α chains of the IgA molecule. The J chain is produced by the same cells that produce the IgA and form the linkage between the two monomeric IgA molecules. The secretory component is synthesized by epithelial cells and is linked to the Fc of the IgA-J chain dimer as it passes through the epithelial cells of the mucosa. Since the dimeric IgA is more stable than the monomeric IgA in the mucous fluids, it is preferred to use dimeric IgA for *in vivo* therapeutic use.

In order to produce the dimeric form of IgA, the myeloma host cell must also be transfected with the gene encoding the J chain, along with the genes for the recombinant α and κ (or λ) chains. The host cell will then produce the dimeric IgA.

The monomeric or dimeric human monoclonal IgA antibodies are probably the most native and the most acceptable foreign antibody molecules that can be introduced to the mucosal surfaces. These molecules are expected to achieve the "immune exclusion" function and prevent allergenic molecules from entering the mucosal tissue. The immune system has evolved such that the complexes formed by the foreign allergenic molecules and the IgA will not be taken up by the mucosal epithelial cells. The immune complexes on the nasal linings are

excreted as the mucous excretion is expelled externally through the nostrils. The immune complexes and other inhaled particles on the mucosal surfaces of the tracheal and bronchial airways will be expelled into the mouth. The mucous that enters the mouth will be mixed with the saliva, swallowed, and digested by the gastrointestinal tract.

In order to achieve better adsorbing and clearing of allergenic molecules from the mucous fluids on the mucosal surfaces, and to prevent any uptake of the complexed allergen by the mucosal epithelial cells, the allergen-specific IgA or its antigen-binding sites, such as $F(ab')_2$, Fab, or Fv, can be conjugated to polymer backbones or microbeads. Allergen-specific IgG and its fragments could also be used in these conjugates. Suitable polymers include dextran, agarose, cellulose, and other polymeric substances that are known to be inert, non-allergenic, and non-immunogenic. After chemical modification to generate active sites, the polymer backbone can provide sites for the coupling of a large number of antibody molecules. The microbeads are preferably in the range of 0.1-10 μm in diameter and made of the polymers mentioned above. Suspensions of these microbeads can be made homogeneous with gentle shaking, and hence are suitable for application to the affected mucosal tissues.

The modes of application for the allergen-specific monoclonal antibodies depends on several factors. For determining whether an allergen-specific preparation is suitable, the patient needs to be diagnosed

to determine whether the particular allergen is a major allergy-inducing molecule for him. If a patient is sensitive to several allergenic molecules, a preparation containing multiple IgA monoclonal antibodies each specific to one of the allergens may be used.

As discussed above, the amount of allergen entering the mucosal surfaces in most cases is very small. However, the mucous secretions are being excreted by the mucosal tissues constantly. This suggests that the solution or suspension containing allergen-specific immunoglobulins (IgA or IgG) should be applied in small quantities but at frequent intervals. It is estimated that for each individual application to the nasal linings, eyes, or ears, amounts of 1-50 μg of immunoglobulin per nostril, eye, or ear will be sufficient. Depending on the individual's rate of secretion from the nose and from the eyes, the preparation may be applied about every two to every six hours. The frequency of application can be reduced if a decongestant is concurrently used. Because the areas of the mucosal surfaces involved in allergic asthma are much larger, more immunoglobulin will need to be applied to these surfaces for asthma treatment.

The preparation can be applied to the nasal linings, eyes, and ears with a dropper, preferably one which delivers 1-2 drops of the solution to each nostril, an eye or an ear. The concentration of the immunoglobulin is in the range of 20 to 1000 μg/ml. The solution can be packaged in 2-5

ml vials and stored in the refrigerator. Once opened, the vial should be used in one week. For asthma treatment, the solution or suspension containing about the same concentration of allergen-specific immunoglobulin can be administered to the lower parts of the airway using a metered dose inhaler.

The allergen-specific IgA and IgG, and the related conjugates with polymers and microbeads, can also be used in manufacturing pure and safe allergenic substances, which can be used in various diagnostic assays and in desensitization immunotherapy. The allergen-specific antibodies can be adsorbed onto an affinity column for purifying the responsible, specific allergens from the crude extracts of allergenic substances, such as ragweeds, grass and tree pollens, house dusts, and cat and dog dander. The purified allergens can be used to make allergen extracts. The purified allergens are better antigens than the crude extracts for diagnostic assays and for desensitization immunotherapy. The purified allergen-specific IgA and IgG can be also used as standards in diagnostic assays for measuring allergen-specific antibodies in the patients receiving immunotherapy. These assays are useful in monitoring the efficacy of the immunotherapy.

Example 1: Selection of blood donors producing IgG specific for *Der p* I

Patients who had a high sensitivity to house dust mites and who have received or are receiving desensitization immunotherapy with mite extract can be identified in an allergy clinic, for example, Allergy Asthma

Immunology Associates of Texas in Houston, TX, directed by Dr. Thomas R. Woehler. A protocol for performing studies on patients' blood samples has been approved by the institutional review board overseeing that clinic. The sera from the patients will be tested for IgG specific for *Der p* I in an immunology clinical laboratory, for example, Reference Laboratory for Dermatology, Allergy, and Clinical Immunology in the Johns Hopkins University School of Medicine (Baltimore, MD), which runs *Der p* I-specific IgG assays routinely. The sera are also assayed for *Der p* I-specific IgG using the ELISA described in **Example 2** below.

The patients with high serum titers of *Der p* I-specific IgG will be recruited as donors of blood. The blood will be used for the isolation of the antigen-specific immunoglobulin-producing B cell clones.

Example 2: Establishing an ELISA for *Der p* I-specific IgG and IgA

The cDNA for *Der p* I is cloned using a routine PCR method from a sample of house mites purchased from Commonwealth Serum Laboratories, Parkville, Australia. The sequence of the cDNA has been published, and is known by those skilled in the art. Chua, K.Y. *et al. J. Exp. Med.* 167: 175-182 (1988). The sequences of the oligonucleotide primers for PCR can be readily designed from the available cDNA sequence. The cDNA can then be expressed in abundant quantities in one of several commercially available systems. A preferred expression system is one that uses the expression vector derived from nuclear polyhedron

protein gene of the insect virus baculovirus (*Autographa californica*). The virus can be grown in the insect cells, sf9 cells, from *Sprodoptera frugiperda*. Luckow, V.A. and Summers, M.D. *Virology* 167: 31 (1989). This expression system has been packaged into a convenient kit (designated "Max Bac Baculovirus Express System") and is sold by Invitrogen (San Diego, CA).

After an adequate quantity of the *Der p* I is prepared, the purified protein will be used as the solid-phase antigen for an ELISA to detect and assay human IgG or IgA in culture supernatants of immortalized B cell clones or transfectomas. A preferred format for the ELISA employs biotin-labeled goat IgG anti-human IgG and goat IgG anti-human IgA, and horseradish peroxidase-conjugated avidin. Harlow,E. and Lane, D. *Antibodies: A Laboratory Manual*. pp. 553-612. Cold Spring Harbor Laboratory (1988). The ELISA may also be used to quantitate *Der p* I-specific IgG in human sera.

Example 3: Making Amb a 1-specific IgA

1. Establishment of cell lines from patient PBLs.

A total of 16 blood samples were collected from 15 patients who had been immunized with allergens including ragweed extracts. Purified PBLs were first immortalized by transformation with EBV and then fusion with mouse myeloma cell line 653. Culture supernatants from the resultant clones were screened by ELISA using plastic coated Amb a 1

protein (prepared by Greer Laboratory to 95% purity) and horseradish peroxidase-conjugated goat anti-human IgG (Fc) antibody. A total of 13 cell lines were expanded and further characterized by subcloning. A single cell subclone AL 16-5.2, secreting Amb a1-specific IgG4,κ antibody was chosen for class-switching to IgA1, by recombinant DNA techniques.

2. Cloning of the AL 16-5.2 heavy and light chain genes.

Total RNA was prepared form the AL 16-5.2 cells and first strand cDNA was prepared using oligo dT primers. When the first strand cDNA was used as the template, and the 5' and 3' κ light chain primers (Table 1) were used in PCR, an amplified band of the expected size was noted. The DNA sequence of several subclones containing this amplified DNA fragment was determined. The nucleotide sequence and its deduced amino acid sequence are shown in SEQ ID NO:1.

When PCR was carried out using oligonucleotide primer sets derived from the leader or framework regions of the human H-chain V genes as the 5' primers, and the oligonucleotide derived from the human Cγ region as the 3' primer, no DNA fragment of the expected size for V_H was amplified.

The V_H gene of AL 16-5.2 cells was cloned by anchored PCR as follows. Double-stranded cDNA of AL 16-5.2 was prepared using oligo NotI-CG1 (Table 1) as the primer. Annealed adaptors (Table 1) were added to both 5' and 3' termini of the cDNA. Adaptors 3' to the C

region were removed by Not I digestion. Digested product was used as the template in PCR with the adaptor primer as the 5' primer and CG2 as the 3' primer (Table I). The reaction mixture was again amplified in a second PCR with oligo ANC as the 5' primer and CG3 as the 3' primer. DNA fragments between 500 and 650 bp in length were purified and subcloned into puc 19. Colonies were hybridized with γ-^{32}p labelled oligo CG4 probe (Table I). Positive clones with full length CDNA inserts were analyzed by DNA sequencing. All four oligonucleotides, NotI-CG1, CG2, CG3, and CG4, were derived from the consensus regions of the four human IgG subclasses. The nucleotide sequence of the V_H gene and its deduced amino acid sequence are shown in SEQ ID NO:2.

Comparison of the deduced AL 16-5.2 L-chain sequence with human V region sequences indicated that the AL 16-5.2 L-chain is a member of the human V_K III subgroup. The AL 16-5.2 H-chain sequence reveals that it belongs to the human V_H III subgroup.

3. Construction of expression vectors.

The AL 16-5.2 V_K gene was inserted into our TB102 hollow vector which contained the Ig promotor, enhancer elements, and the leader sequences derived from the H-chain locus of the high-producing murine hybridoma TB102 cells. DNA fragments containing the promotor, enhancer, and the leader of the TB102 H-chain locus, and the coding sequence of the AL 16-5.2 V_K gene was cloned into a vector containing

the human Cκ region and the neo gene to give the κ light chain expression plasmid, pSV neo 16-5.2 V_K - h C_K. Similarly, the DNA fragment containing the coding sequence of the AL 16-5.2 V_H gene and the TB102 flanking sequences was cloned into a vector containing human Cγ1 region and the gpt gene to give the γ heavy chain expression plasmid, pSV2 gpt 16-5.2V_H-h Cγ1.

4. Generation of cell lines secreting Amba1-binding IgA.

Linearized plasmid DNAs of both κ light chain and γ heavy chain expression vectors were introduced into mouse myeloma NSO cells by electroporation. Transfected cells were selected in growth medium containing 0.15 mg/ml G418 0.1 μg/ml mycophenolic acid, and 50 μg/ml xanthine for both κ and γ-chain plasmid DNA uptake. Supernatants of transfectants were tested for the production of Amb a 1-binding IgA by ELISA using plastic-coated Amb a1 and horseradish peroxidase-conjugated goat anti-human IgA antibody. Culture supernatants from several cell lines will be used to prepare purified IgA for further analysis.

Table 1. Design of synthetic oligonucleotide primers for (A) light chain and (B) heavy chain cloning

(A) Kappa Light Chain

5' primer (SEQ ID NO:3)

GGGAATTCATGGACATG(AG)(AG)(AG)(AGT)(CT)CC(ACT)(ACG)G(CT)(GT)CA(CG)CTT *

* Nucleotides in parenthesis indicate that either nucleotide can be present, due to degeneracy

3' primer (SEQ ID NO:4) CCAAGCTTCATCAGATGGCGGGAAGAT

(B) Gamma Heavy Chain

Annealled adaptor (SEQ ID NO:5) AATTCGAACCCCTTCG

Adaptor primer (SEQ ID NO:6) AATTCGAACCCCTTCG

NotI-CG1 (SEQ ID NO:7) TGCGGCCGCTGCTGAGGGAGTAGA

CG2 (SEQ ID NO:8) AGGTGTGCACGCCGCTGGTC

CG3 (SEQ ID NO:9) CAAGCTTCCACGACACCGTCACCGG

CG4 (SEQ ID NO:10) GCCCTGGGCTGCCTGGTCAAGGACTACTTC

It should be understood that the terms, expressions, and examples herein are exemplary only and not limiting, and those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. All such equivalents are intended to be

encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) Applicant: Chang, Tse Wen
(ii) Title of Invention: ALLERGEN-SPECIFIC IgA MONOCLONAL ANTIBODIES AND RELATED PRODUCTS FOR ALLERGY TREATMENT
(iii) Number of Sequences: 10
(iv) Correspondence Address:
(A) Addressee: Tanox Biosystems, Inc.
(B) Street: 10301 Stella Link Rd.
(C) City: Houston
(D) State: Texas
(E) Country: USA
(F) ZIP: 77025
(v) Computer Readable Form
(A) Medium Type: 3.5" Hi Density Diskette
(B) Computer: IBM PS/2
(C) Operating System: DOS, Version 3.30
(D) Sofware: Wordperfect 5.1
(vi) Current application data:
(A) Application Number:
(B) Filing date:
(C) Classification: unknown
(vii) Prior Application Data:
(A) Application Number:
(B) Filing Date:
(viii) Attorney/Agent Information:
(A) Name: Mirabel, Eric P.
(B) Registration Number: 31,211
(C) Reference/Docket Number: TNX92-3
(ix) Telecommunication Information:
(A) Telephone: 713-664-2288
(B) Telefax: 713-664-8914
(2) Information for SEQ ID NO:1:
(i) Sequence Characteristics:
(A) Length: 325 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double stranded
(D) Topology: Linear

(xi) Sequence Description: SEQ ID NO:1

```
GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT  42
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser
                  5                  10

CCA GGG GAA AGA GCC ACC CTC TCC TGC AGG GCC AGT CAG ACT  84
Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Thr
15                  20                  25
```

```
GTT AGC AGC AAC TAC TTA GCC TGG TAC CAG CAC AAA CCT GGC  126
Val Ser Ser Asn Tyr Leu Ala Trp Tyr Gln His Lys Pro Gly
     30                  35                  40

CAG GCT CCC AGG CTC CTC ATC TAT GCT ACA TCC ATA AGG TCA  168
Gln Ala Pro Arg Leu Leu Ile Tyr Ala Thr Ser Ile Arg Ser
         45                  50                  55

TCT GGC ATC CCA GAC AGG TTC ACT GGC AGT GGG TCT GGG ACA GAC  213
Ser Gly Ile Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
            60                  65                  70

TTC ACT CTC ACC ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG  258
Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val
            75                  80                  85

TAT TAC TGT CAG CAG TTT CGT AAC TCA CAG TGG ACG TTC GGC CAA  303
Tyr Tyr Cys Gln Gln Phe Arg Asn Ser Gln Trp Thr Phe Gly Gln
            90                  95                  100

GGG ACC AAG GTT GAA ATC AAA C 325
Gly Thr Lys Val Glu Ile Lys Arg
            105
```

(2) Information for SEQ ID NO:2:
(i) Sequence Characteristics:
(A) Length: 376 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double stranded
(D) Topology: Linear
(xi) Sequence Description: SEQ ID NO:2

```
GAG GAG CAG GTG GTG GAG TCT GGG GGA GGC CAG GTC AAG CCG GGG 45
Glu Glu Gln Val Val Glu Ser Gly Gly Gly Gln Val Lys Pro Gly
                  5                  10                  15

GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC AAT TTC ACC 90
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Phe Thr
                 20                  25                  30

AGC TAC AGC ATG AAC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG 135
Ser Tyr Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

GAG TGG GTC GCA TCA ATT ACG AGC AGA AGT ACT TAC ACC TTC TAC 180
Glu Trp Val Ala Ser Ile Thr Ser Arg Ser Thr Tyr Thr Phe Tyr
                 50                  55                  60

GCA GAC TCA TTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAC GCC 225
Ala Asp Ser Leu Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
                 65                  70                  75

AAG AAC TCT CTC TTT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC 270
Lys Asn Ser Leu Phe Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90


ACG GCT GTT TAT TAC TGT GCG AGA GTA TTG ATC TTT GAC TCA AAC 315
Thr Ala Val Tyr Tyr Cys Ala Arg Val Leu Ile Phe Asp Ser Asn
                 95                 100                 105

AAC TTT TAT TAC TAC TAC ATG GAC GTC TGG GGC AAA GGG ACC ACG 360
Asn Phe Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr
                110                 115                 120

GTC GCC GTC TCC TCA G 376
Val Ala Val Ser Ser
               125
```

(2) Information for SEQ ID NO:3:
(i) Sequence Characteristics:
(A) Length: 35 nucleotides
(B) Type: nucleic acid

(C) Strandedness: single stranded
(D) Topology: Linear
(xi) Sequence Description: SEQ ID NO:3
GGGAATTCAT GGACATGAAA ACCCAAGCGC ACCTT 35

(2) Information for SEQ ID NO:4:
(i) Sequence Characteristics:
(A) Length: 27 nucleotides
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: Linear
(xi) Sequence Description: SEQ ID NO:4

CCAAGCTTCA TCAGATGGCG GGAAGAT 27

(2) Information for SEQ ID NO:5:
(i) Sequence Characteristics:
(A) Length: 16 nucleotides
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: Linear
(xi) Sequence Description: SEQ ID NO:5

AATTCGAACC CCTTCG 16

(2) Information for SEQ ID NO:6:
(i) Sequence Characteristics:
(A) Length: 24 nucleotides
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: Linear
(xi) Sequence Description: SEQ ID NO:6

TGCGGCCGCT GCTGAGGGAG TAGA 24

(2) Information for SEQ ID NO:7:
(i) Sequence Characteristics:
(A) Length: 24 nucleotides
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: Linear
(xi) Sequence Description: SEQ ID NO:7

TGCGGCCGCT GCTGAGGGAG TAGA 24

(2) Information for SEQ ID NO:8:
(i) Sequence Characteristics:
(A) Length: 20 nucleotides
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: Linear

(xi) Sequence Description: SEQ ID NO:8

AGGTGTGCAC GCCGCTGGTC 20

(2) Information for SEQ ID NO:9:
(i) Sequence Characteristics:
(A) Length: 25 nucleotides
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: Linear
(xi) Sequence Description: SEQ ID NO:9

CAAGCTTCCA CGACACCGTC ACCGG 25

(2) Information for SEQ ID NO:10:
(i) Sequence Characteristics:
(A) Length: 30 nucleotides
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: Linear
(xi) Sequence Description: SEQ ID NO:10

GCCCTGGGCT GCCTGGTCAA GGACTACTTC 30

What is Claimed is:

1. A pharmaceutical suspension or solution of allergen-specific IgA, suitable for application to mucosal tissues.

2. A pharmaceutical suspension or solution of claim 1, in which the allergen is *Amb a* I, *Der p* I, *Der f* I, *Can f* I, or *Fel d* I.

3. A pharmaceutical suspension or solution of claim 1, further including water and preservatives.

4. A pharmaceutical solution of claim 3, further including surfactants.

5. A conjugate comprising a polymer backbone and a plurality of covalently conjugated allergen-specific binding molecules.

6. A conjugate of claim 5, in which the polymer is dextran, agarose, or cellulose.

7. A conjugate of claim 5, in which the allergen-binding molecules are allergen-specific IgA or IgG, or the $F(ab')_2$, Fab, or Fv fragments thereof.

8. A conjugate of claim 5, in which the allergen is *Amb a* I, *Der p* I, *Der f* I, *Can f* I, or *Fel d* I.

9. A pharmaceutical preparation comprising a polymer backbone and a plurality of covalently conjugated allergen-specific binding molecules.

10. The pharmaceutical preparation of claim 9 further including water, surfactants and preservatives.

INTERNATIONAL SEARCH REPORT

In' ational application No.
PCT/US93/12501

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/35; C07K 15/28
US CL :424/85.8; 530/388.5, 388.85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.8; 530/388.5, 388.85

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, MEDLINE, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,226,853 (MARSH) 07 October 1980, col. 1, lines 23-29.	1-4
Y	US, A, 5,026,545 (SAINT-REMY et al) 25 June 1991, col. 18, lines 58-59.	1-4
Y	J. Exp. Med., Volume 167, issued January 1988, K. Y. Chua et al., " Sequence Analysis of cDNA Coding for a Major House Dust Mite Allergen, *Der p* 1", pages 175-182, especially page 175.	1-4
Y	J. Allergy Clin. Immunol., Volume 87, No. 1, part 1, issued January 1991, S. Lau-Schadendorf et al., "Short-Term Effect of Solidified Benzyl Benzoate on Mite-allergen concentrations in House Dust", pages 41-47, especially page 41.	1-4
Y	The Journal of Immunology, Volume 146, No. 10, issued 15 May 1991, J. F. Bond et al., "Multiple *Amb a* I Allergens Demonstrate	1-4

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 FEBRUARY 1994	Date of mailing of the international search report 14 MAR 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer CHRISTINA CHAN Jill Warden for Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

In. ational application No.
PCT/US93/12501

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Allergy Clin. Immunol., Volume 87, No. 6, issued June 1991, H. de Groot et al., "Affinity Purification of a Major and a Minor Allergen from Dog Extract: Serologic Activity of Affinity-Purified Can f I and of Can f I-Depleted Extract", pages 1056-1065, especially page 1056.	1-4
Y	Proc. Natl. Acad. Sci. USA, Volume 88, issued November 1991, J. P. Morgenstern et al., "Amino Acid Sequence of Fel dI, the Major Allergen of the Domestic Cat: Protein Sequence Analysis and cDNA cloning", pages 9690-9694, especially page 9690.	1-4

INTERNATIONAL SEARCH REPORT

In' ıtional application No.
PCT/US93/12501

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: -
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

I. Claims 1-4, drawn to a pharmaceutical suspension or solution, classified in Class 424/85.8.

II. Claims 5-10, drawn to a conjugate and a pharmaceutical preparation, classified in Classes 530/391.1 and 424/85.91

The pharmaceutical preparation comprising the antibody in Group I would not suggest the conjugate and the pharmaceutical preparation comprising the conjugate in Group II. Groups I and II are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-4

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.